Steady-state measurement of NO and CO lung diffusing capacity on moderate exercise in men

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Borland, Colin, Bryan Mist, Mariella Zammit, and Alain Vuylsteke. Steady-state measurement of NO and CO lung diffusing capacity on moderate exercise in men. J Appl Physiol 90: 538–544, 2001.—Using a rapidly responding nitric oxide (NO) analyzer, we measured the steady-state NO diffusing capacity (DLNO) from end-tidal NO. The diffusing capacity of the alveolar capillary membrane and pulmonary capillary blood volume were calculated from the steady-state diffusing capacity for CO (measured simultaneously) and the specific transfer conductance of blood per milliliter for NO and CO, calculated from end-tidal (assumed alveolar) CO2, mixed expired CO2 and mixed expired CO, was 46.9 ± 12.8 ml min⁻¹ Torr⁻¹, NO dead space = (VT × FENO − VT × FANO)/(FENO − FANO) = 209 ± 88 ml, where VT is tidal volume and FENO, FANO, and VNOS are mixed expired, inhaled, and alveolar NO concentrations, respectively. We used the Bohr equation to estimate CO2 dead space from mixed exhaled and end-tidal (assumed alveolar) CO2 = 430 ± 136 ml. Predicted anatomic dead space = 199 ± 22 ml. Membrane diffusing capacity was 333 and 166 ml min⁻¹ Torr⁻¹ for NO and CO, respectively, and pulmonary capillary blood volume was 140 ml. Inhalation of repeated breaths of NO over 80 s did not alter DLNO at the concentrations used.

alveolar capillary gas diffusion; dead space; membrane diffusing capacity; lung capillary blood volume

THEORETICALLY, nitric oxide (NO) is the ideal gas to study lung diffusion because it is poorly soluble in water and, because of its very rapid and virtually irreversible reaction with Hb, its uptake is independent of pulmonary capillary blood flow and rate of chemical reaction (6). Our laboratory (4) originally developed a single-breath method for measuring lung NO diffusion (DLNOss) during tidal breathing at rest. By including CO in the inspired mixture, it is possible to simultaneously measure the DLCOss. From knowledge of the water solubility and molecular weight of the two gases and by assuming that Roughton and Forster’s model also applies to NO lung transfer, our laboratory (3) and others (8) have estimated the Dm and Vc from a single breath. The availability of rapidly responding and sensitive NO analyzers now allows estimation of alveolar NO concentrations from end-expiratory samples and hence measurement of steady-state DLNO (DLNOss) during tidal breathing using safe and stable NO concentrations.

These measurements are important for several reasons. NO is produced in high concentration (11) in the nose and taken up in the lung, so steady-state NO gas transfer takes place during everyday life and knowledge of DLNO is essential for understanding normal NO metabolism. Measuring diffusing capacity in animals and on maximal exercise in humans is easier using a steady-state method compared with a single-breath technique. Using Roughton and Forster’s method for obtaining Dm and Vc from DLCO during steady-state breathing at two or more O2 tensions is likely to overestimate the O2-dependent resistance (1/|6 Vc|) due to hyperoxia increasing regional inhomogeneity in diffusing capacity. Using DLNO and DLCO at a single, physiological value for Pco2 obviates this problem. Exhaled NO is being investigated as a test for inflammatory markers in lung disease. An increase in FENO could result from increased NO production or from reduced DLNO (10). Ill or ventilated patients will be unable to perform single exhalations from total lung capacity, so knowing DLNOss is important in interpreting values for FENO derived from tidal breathing measurements. Finally, inhaled NO is being used therapeutically to treat acute respiratory distress syndrome and pulmonary hypertension. NO causes ultrastructural oxidant lung injury when inhaled in concentrations of 6 ppm for 6 wk (12), and its safety depends on its fast removal into the pulmonary capillary blood before oxidation to nitrogen dioxide or other toxic products can occur. If patients have reduced DLNOss, then toxicity may be enhanced and the inhaled NO concentration may need to be reduced.

For these reasons, we have made combined DLNOss and DLCOss measurements in healthy volunteers by adapting the method of Bates et al. (1).

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Glossary

- **NO**: Nitric oxide (parts per billion (ppb))
- **DL**: Lung diffusing capacity
- **DLNO**: Diffusing capacity for NO
- **DLCO**: Diffusing capacity for CO
- **DLNOSS**: Steady-state diffusing capacity for NO
- **DLCOSS**: Steady-state diffusing capacity for CO
- **DLCOSB**: Single-breath diffusing capacity for CO
- **FACO2**: Alveolar CO2 concentration (%)
- **FANO**: Alveolar NO concentration (ppb)
- **FECO**: Mixed exhaled CO concentration (%)
- **FINO**: Inhaled NO concentration (ppb)
- **FNO**: Mixed exhaled NO concentration (ppb)
- **V˙O2**: O2 uptake (l/min)
- **Vc**: Pulmonary capillary blood volume (ml)
- **PcO2**: Partial pressure of O2 in pulmonary capillaries (Torr)
- **VT**: Tidal volume (liters)
- **VA**: Alveolar volume (liters)
- **RR**: Respiratory rate (min⁻¹)
- **Pb**: Barometric pressure (Torr)
- **θNO**: Specific transfer conductance of blood per milliliter for NO (ml·min⁻¹·Torr⁻¹·ml⁻¹)
- **θCO**: Specific transfer conductance of blood per milliliter for CO (ml·min⁻¹·Torr⁻¹·ml⁻¹)
- **FACO2**: Alveolar CO2 concentration (%)
- **FECO**: Mixed exhaled CO concentration (%)
- **FNO**: Alveolar NO concentration (ppb)
- **VO2**: O2 uptake (l/min)
- **Hb**: Concentration of hemoglobin in venous blood (g/dl)
- **COHb**: Concentration of carboxyhemoglobin in venous blood (%)
- **methHb**: Concentration of methemoglobin in venous blood (%)

**METHODS**

**Subjects**

Nine nonsmoking male subjects with no history of lung disease volunteered for the study. All signed consent forms approved by the Huntingdon district ethics committee, who also gave approval for this study. Their characteristics and DLCOSS measured by the standard technique at rest (6) are listed in Table 1. All subjects pursued a moderately active lifestyle.

**General Method**

On a daily basis, temperature and Pb were measured by use of a metabolic cart (2900Z, Sensor Medics EME, Brighton, Sussex, England) calibrated against a mercury thermometer and the local meteorological office, respectively. Pb was corrected for saturated vapor pressure by reference to a table (6). Each subject performed one complete maneuver. Background atmospheric NO, CO2, and CO were measured. Each subject sat breathing air on an electronically braked bicycle ergometer (Sensor Medics 800S) and started to cycle, gradually building up speed until he achieved the desired level of moderate exercise (~1 l/min V˙O2). Once steady state was achieved, exhaled air was collected for a 2-min period to measure NO and CO back tension, and an 80-s record was also taken of intrabreath NO and CO2 recording at the lips by arranging a fine-bore cannula within the mouthpiece to ensure zero dead space. They were then switched to a mixture of ~5,000 ppb NO and 0.1% CO in air stored in a 200-liter Douglas bag (PK Morgan, Gillingham, Kent, UK) prepared immediately before each replicate. A continuous 2-min collection of exhaled air was made from a similar Douglas bag attached to the exhaled port of the metabolic cart. Vr on a breath-by-breath basis, RR, and V02 were recorded during this time from the metabolic cart. Volume was calibrated by using a 3-liter syringe. The O2 analyzer of the metabolic cart was calibrated using three different O2 concentrations (16%, 21%, and 26%). Immediately before and immediately after the period of exercise, the inspirate bag was analyzed for NO and CO.

**Gas Analyses**

NO and CO2 were analyzed by using a rapidly responding instrument (Logan Research LR 2000, Rochester, UK) that directs the sample through a rapidly reacting infrared CO2 analyzer to a chemiluminescent NO analyzer with a small (~10 ml) reaction chamber. The NO analyzer has four separate analysis modes for quantifying endogenous NO produced 1) through the nose, 2) during tidal breathing, and 3) during a maximal single exhalation and, finally, 4) a mode for analyzing exhaled concentrations during therapeutic use of NO. For this study, the instrument was set in “therapeutic” mode and calibrated using NO-free compressed air and 4,000 ppb and 80,000 ppb NO (manufacturer’s certificate of analysis; BOC gases, Worsley, Manchester, UK). The CO2 analyzer

**Table 1. Physical characteristics of subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>DLCOSS, ml·min⁻¹·Torr⁻¹</th>
<th>Anatomic Dead Space, ml</th>
<th>Oxygen Uptake, l/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>185</td>
<td>80</td>
<td>221</td>
<td>37.2</td>
<td>1.35</td>
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<td>2</td>
<td>43</td>
<td>187</td>
<td>88</td>
<td>237</td>
<td>37.2</td>
<td>1.14</td>
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<td>3</td>
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<td>180</td>
<td>70</td>
<td>173</td>
<td>42.8</td>
<td>1.45</td>
</tr>
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<td>4</td>
<td>37</td>
<td>168</td>
<td>68</td>
<td>187</td>
<td>24.4</td>
<td>1.07</td>
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<tr>
<td>5</td>
<td>32</td>
<td>182</td>
<td>61</td>
<td>166</td>
<td>33</td>
<td>0.98</td>
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<td>33</td>
<td>165</td>
<td>80</td>
<td>209</td>
<td>32</td>
<td>1.48</td>
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<td>7</td>
<td>31</td>
<td>183</td>
<td>83</td>
<td>214</td>
<td>43.1</td>
<td>1.39</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
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<td>75</td>
<td>188</td>
<td>30.6</td>
<td>1.44</td>
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<tr>
<td>9</td>
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<td>78</td>
<td>200</td>
<td>37.2</td>
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<td>Mean ± SD</td>
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<td>178 ± 8</td>
<td>76 ± 8</td>
<td>199 ± 22</td>
<td>35.3 ± 5.6</td>
<td>1.32 ± 0.20</td>
</tr>
</tbody>
</table>

DLCOSS, single-breath diffusing capacity for CO.
was calibrated using O₂ (CO₂ free), 5% and 6% CO₂. To ensure linearity of these two analyzers, a serial dilution of a mix of NO and CO₂ over the working range was performed. To measure the response time, a rubber balloon containing 2,660 ppb NO and 2.7% CO₂ was burst by pinprick.

CO was analyzed by using the infrared analyzer of a standard gas transfer apparatus (Transfertest, PK Morgan, Chatham, Kent, UK). Linearity was tested by serially diluting a standard CO-He mix of 6% He and 0.1% CO in air to generate a 25-point plot of CO vs. He.

**Blood Analyses**

Venous blood was sampled immediately before each experiment and analyzed within 2 h for Hb, metHb, and COHb using an automated spectrophotometer (IL282, Instrument Laboratories, Cupertino, CA).

**Safety Precautions**

The exercise laboratory was kept well ventilated at all times. The stock NO cylinder (1,000 ppm in nitrogen) was kept securely fastened to the purpose-built trolley at all times. The inspired NO concentration in the Douglas bag was checked before inhalation and was continuously monitored by breath during the experiment.

**Calculations**

*Alveolar NO and CO₂.* The NO, CO₂, and time (in s) readings were downloaded as Excel spreadsheet files (Microsoft) from the Logan analyzer using the “datadump facility.” For each individual, four files were created: mixed exhaled (breathing air on exercise), mixed exhaled (breathing NO and CO on exercise), inhaled (breathing NO and CO on exercise), and breath by breath (breathing NO and CO on exercise). For the inhaled file, the concentrations were derived as the mean of the column of readings. For the breath-by-breath files, X-Y plots of NO as a function of time were drawn using a spreadsheet charting program (Works 3.1, Microsoft). The FₐCO₂ was taken as one-third of the way along the alveolar plateau (Fig. 1) (5).

For NO, the alveolar phase was identified visually as a stepwise reduction in the rate of fall of NO concentration with time (Fig. 2), a line of best fit was drawn, and the estimated alveolar concentration one-third of the way along was taken as FₐNO. FₐCO₂ and FₑNO were taken as the mean of the column of readings from the exhaled bag.

**Dead space calculations.** Anatomic dead space was estimated as 2.2 × weight (kg) + age in yr (6). CO₂ dead space (6) was calculated from

\[
V_d/V_T = (F_{aCO_2} - F_{eCO_2})/F_{aCO_2}
\]  

(1)

NO dead space was calculated as follows

\[
V_D = (V_T \cdot F_{eNO} - V_T F_{aNO})/(F_{aNO} - F_{eNO})
\]  

(2)

This rearranges to yield

\[
V_D = V_T \times (V_T - V_D) \times RR \times (F_1 - F_e)/(F_e \times V_T - F_1 \times V_D) \times (P_B - 47)
\]  

(3)

**Diffusing capacity calculations.** We calculated DlCO and DlNO by using estimated anatomic dead space [(a) DlCO I in Bates et al. (1)] and also DlCO using CO₂ dead space [(e) in Ref. 1]

\[
D_L = V_T \times (V_T - V_D) \times RR \times (F_1 - F_e)/(F_e \times V_T - F_1 \times V_D) \times (P_B - 47)
\]  

(4)

Equation 3 was calculated using the predicted anatomic dead space for both gases and, for CO₂, the CO₂ dead space (Eq. 1).

In addition, for NO, because we had a rapidly reacting NO analyzer, we adapted Bates et al.’s method using end-tidal concentrations [(c) DlCO II in their paper] for NO and used end-tidal FₑNO from the expired NO curve (Fig. 2) as an estimate of FₐNO

\[
D_L = V_T \times RR \times (F_{eNO} - F_{aNO})/(F_A \times (P_B - 47))
\]  

There were thus two estimates of DlCO and DlNO per subject.

For dead space and Dl calculations, the respiratory quotient was assumed to be 1 (see Results).

**Calculation of Dm and Vc.** Calculations were made as follows

\[
D_{mNO} = (\theta_{NO} - 2 \cdot \theta_{CO})/\theta_{NO}/D_{lNO} - \theta_{CO}/D_{lCO}
\]  

(5)

\[
D_{mCO} = D_{mNO}/2
\]  

(6)

\[
V_c = 1/(\theta_{CO}/D_{lCO} - \theta_{CO}/D_{mCO})
\]  

(7)
For derivation of these equations, see Ref. 4. The value of $\theta_{NO}$ (5) was taken as $4.5 \text{ ml\cdot min}^{-1} \cdot \text{Torr}^{-1} \cdot \text{ml}^{-1}$ (1,500 mmol$^{-1} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \text{l}^{-1}$) and $\theta_{CO}$ (7) calculated from $1/\theta_{CO} = 1.3 \times 10^{-3} + 4.1 \times 10^{-5} (P_{CO})$ min$^{-1}$ Torr. $P_{CO}$, was taken as 100 Torr. For both CO and NO, correction for Hb was made by multiplying $\theta$ by Hb $\times (1 - COHb - metHb)/14.6$.

For calculation of $D_{m,CO}$, the assumption (8) is made that $\theta_{NO}$ is infinity

$$D_{m,CO} = D_{L,NO}/2 \quad (8)$$

and $V_c$ was obtained by inserting this value for $D_{m,CO}$ into Eq. 7.

**Statistical Analysis**

The paired $t$-test was used to compare the calculated NO dead space to the predicted anatomic dead space and to compare $D_{L,NO}$ calculated from $F_{ANO}$ dead space and to compare $D_{L,NO}$ calculated from $F_{ANO}$ predicted anatomical dead space. Values are given as means $\pm SD$.

**RESULTS**

**Performance of Analyzers**

The NO-6CO analyzer was linear to serial dilution over the working range down to NO concentrations below 250 ppb and CO below 0.55% when departure from linearity occurred; these were well above the alveolar and mixed expired concentrations. The minimum detectable concentration of NO was 60 ppb and CO 0.3% in therapeutic mode. The response time to half signal after balloon burst was 160 ms for CO and 400 ms for NO; the CO$_2$ column was therefore “cut and pasted” down by 240 ms in the spreadsheet to synchronize concentration data for the two gases. A plot of CO and He was linear with intercept zero over the working range down to NO concentrations below 250 ppb and CO 2 below 0.55% when departure from linearity occurred; these were well below the alveolar and mixed expired concentrations.

**Subject Data**

Table 1 illustrates the nine subjects’ physical characteristics.

**Measured and Derived Variables**

Mean $V_{O2}$ was $1.3 \pm 0.2 \text{ l/min}$. Mean respiratory quotient (RQ) was 0.96 $\pm 0.06$. No loss of NO or CO occurred from the inspired bag. There was no detectable (i.e., <60 ppb) background NO in the laboratory atmosphere or in the exhaled breath sample for any subject with the instrument in the therapeutic mode. The breath-by-breath X-Y plots of exhaled concentration and time are shown in Fig. 1 for CO$_2$ and Fig. 2 for NO. The NO plot comprises a peak of inhaled NO, a steep fall representing dead space, and then a shallower alveolar slope. The mean inhaled, mean mixed exhaled, and alveolar NO were $5,218 \pm 1,675, 1,402 \pm 381$, and $942 \pm 250$ ppb, respectively.

The calculated CO$_2$ and NO dead spaces are shown in Table 2. The NO dead space is significantly different ($P < 0.001$) from the CO$_2$ dead space but not from the anatomic dead space. Calculated $D_{L,CO}$ and $D_{L,NO}$ together with $D_m$ and $V_c$ are shown in Tables 2 and 3. There are no significant differences between the two estimates of $D_{L,NO}$. $D_{L,NO}$ appears constant over 30 s of observation (Fig. 3).

**DISCUSSION**

**Major Findings**

Estimates of dead space. The NO dead space is strikingly similar to the predicted anatomical dead space but significantly lower, on average about one-half, than the CO$_2$ dead space. However, the estimate of NO dead space is less precise because the alveolar slope for NO is

![Fig. 3. Ratio of steady-state diffusing capacity for NO ($D_{L,NO}$) to mean $D_{L,NO}$ for the 9 subjects over ~30 s. $D_{L,NO}$ was calculated by using mixed exhaled NO and breath-by-breath alveolar CO$_2$ concentration.](image-url)

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**Table 2. Dead space and $D_l$ estimates**

<table>
<thead>
<tr>
<th>Subject</th>
<th>CO$_2$ Dead Space, ml</th>
<th>NO Dead Space, ml</th>
<th>$D_{L,CO}$ Using Anatomic Dead Space, ml$^{-1}$ min$^{-1}$ $\cdot$ Torr$^{-1}$</th>
<th>$D_{L,CO}$ Using CO$_2$ Dead Space, ml$^{-1}$ min$^{-1}$ $\cdot$ Torr$^{-1}$</th>
<th>$D_{L,NO}$ Using Anatomic Dead Space, ml$^{-1}$ min$^{-1}$ $\cdot$ Torr$^{-1}$</th>
<th>$D_{L,NO}$ Using Alveolar NO, ml$^{-1}$ min$^{-1}$ $\cdot$ Torr$^{-1}$</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>347</td>
<td>145</td>
<td>36.2</td>
<td>38</td>
<td>211.9</td>
<td>184.1</td>
</tr>
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<td>644</td>
<td>420</td>
<td>62.2</td>
<td>59</td>
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<tr>
<td>Mean ± SD</td>
<td>430 ± 136</td>
<td>209 ± 88</td>
<td>42.3 ± 10.6</td>
<td>46.9 ± 12.8</td>
<td>182.6 ± 26.7</td>
<td>205.7 ± 71.8</td>
</tr>
</tbody>
</table>

$D_{L,CO}$: diffusing capacity for CO; $D_{L,NO}$: diffusing capacity for NO.
steeper. Clearly, NO uptake is occurring within the alveolar dead space. NO is a reactive gas particularly in solution, and this reaction could be with groups other than Hb in the lining of unperfused alveoli. Heller and Schuster (9), however, discounted a chemical reaction of NO with lung tissue during single-breath $D_{lNO}$ measurements in rabbits because there was no variation in $D_{lNO}$ either with duration of breath hold or with $F_{INO}$. Their results are in agreement with our single-breath observations of a semilog decline in $F_{ANO}$ with intercept 1 in two human subjects (4) and identical values of $D_{lNOss}$ at $F_{INO}$ of 0.75 ppm and 60 ppm (Borland C and Cox Y, unpublished observations). A more plausible explanation is as follows: the physiological dead space exceeds the anatomic dead space because some alveoli have a ventilation-perfusion ratio approaching infinity. Such alveoli and their associated capillaries will secrete minimal CO2. On the other hand, $D_{lNO}$ is diffusion limited, independent of capillary blood flow, and (if $ONa$ approaches infinity) independent of capillary blood volume. Uptake of NO would therefore occur even in these alveoli, and the NO dead space would correspond to the anatomic dead space.

**Values for $D_{lNO}$** The values for $D_{lNO}$ using the predicted anatomic dead space and the dead space calculated from $F_{ANO}$ are similar (Table 2). The higher mean value for $D_{lNO}$ calculated from $F_{ANO}$ is largely due to subject 3, who had wide between-breath variation in $V_T$ and $F_{ANO}$. The average $D_{lNOss}$ of $\sim 200$ ml·min⁻¹·Torr⁻¹ measured here on moderate exercise exceeds the single-breath value measured at rest (125 ml·min⁻¹·Torr⁻¹) by $\sim 70$ ml·min⁻¹·Torr⁻¹ (4). This is at first surprising because steady-state measurements are made at $V_T$ rather than total lung capacity and $D_{lNOss}$ is highly lung volume dependent (4), falling by 45 ml·min⁻¹·Torr⁻¹ with a reduction in $V_a$ from 7 to 4 liters. It is to be noted that it is the volume that the inhaled NO is distributed into rather than the inhaled volume that is important here. During steady-state breathing, the NO will be distributed into a volume of functional residual capacity plus $V_T$, whereas during a single breath it will be distributed into $V_a$. The difference between these volumes is perhaps only 1–2 liters. However, we also noted a rise in $D_{lNOss}$ with exercise to 210 ml·min⁻¹·Torr⁻¹ at 1–2 l/min $V_o_2$, so the overall value could be little changed. These average values therefore may be used to interpret values for alveolar partial pressure of NO in models of NO production (10).

**Values for $DlCO$, $Dm$, and $Vc$.** The mean $DlCOss$ on moderate exercise exceeds $DlCOss$ measured at rest by $\sim 5$–10 ml·min⁻¹·Torr⁻¹. Our previous mean value for $DlCOss$ at 1.4 l/min $V_o_2$ in three individuals was 48 ml·min⁻¹·Torr⁻¹, so again it is possible that the discrepancy is due to exercise. Other workers have found $DlCOss$ to be less than $DlCOss$ (13). $DmCOss$ is close to our previous value for $DmCOss$ (316 ml·min⁻¹·Torr⁻¹). However, $Vc$ is about three times as large (56 ml single breath) (3). It is possible to use Eq. 7 to calculate $Vc$ on exercise from our previous combined single-breath data (4), and a figure of 101 ml is obtained. In contrast, $DmCOss$ is 343 ml·min⁻¹·Torr⁻¹. It would appear that during tidal breathing on exercise $Vc$ is one to two times that which is measured during apnea at total lung capacity on exercise. One explanation is that the capillaries are flattened when the alveoli are fully inflated and stretched. Alternatively, maximal inspiration recruits alveoli that are normally less well ventilated, less well perfused, and hence of lower $Vc$. All these processes have little effect on $Dm$ because it is probable that NO uptake is largely independent of $Vc$.

**Variation in $DlNO$ between breaths.** It was not possible to perform a complete breath-by-breath analysis for $DlNOss$. Although we could measure $F_{ANO}$ on a breath-by-breath basis and inhaled NO would not have varied between breaths, it was not possible to calculate the mixed exhaled NO on a breath-by-breath basis. To do so would have needed exhaled flow or volume averaging of the exhaled NO signal, which is not possible with the analyzer in therapeutic mode. However, calculating $DlNOss$ by using $F_{ENO}$ and breath-by-breath $F_{ANO}$ does not show any alteration of $DlNOss$ with duration of inhalation or number of breaths (Fig. 3). Despite NO being vasoactive, there appears to be no evidence that breathing it alters the measurement of $DlNOss$.

**Critique of Method**

As with $DlCOss$, $DlNOss$ suffers from the inherent inaccuracy of estimating $F_{ANO}$. We found a steep alveolar phase for exhaled NO (Fig. 2), and the place on the curve chosen as a representative value for $F_{ANO}$ inevitably influenced $DlNOss$ to a great extent. $DlNOss$ measurements are therefore more reproducible (4). $DlNOss$ is more practical for exercise measurements, and indeed we studied $DlNOss$ on exercise because at rest $DlCOss$ is greatly affected by regional inhomogeneity in diffusing capacity, and we anticipated similar problems with $DlNOss$. It would have been of interest to monitor $DlNOss$ continuously during increasing exercise and to have several replicates per subject. However, this would have involved exposure to 5,000 ppb NO for many minutes over several days, and we thought that this could be unsafe given that there is uncertainty regarding the safe exposure concentration for NO. This concentration was chosen because it is stable in air but sufficiently high for interference from endogenous NO not to be a problem. The steady-state method is more applicable to use during artificial ventilation in patients and for animal work.

**Comparison With Other Work**

All three groups who have made $DlNO$ measurements have applied the classic Roughton and Forster model \(1/Dl = 1/Dm + 1/(Dm/Vc)\) originally derived for CO transfer to NO transfer, although each group has made different assumptions, leading to differing values for $Dm$ and $Vc$. Guennard et al. (8) assumed that, because the rate of reaction of NO with Hb is extremely rapid compared with the membrane conductance ($Dm$), $DlNO$ approximates to $DmNO$ and is therefore twice $DmCO$ because the ratio of
The ratio of DLNO to DLCO varies between 3.9 and 145. It assumes that previously found some O2 variability of DLNO over the situation of NO and O2. However, our laboratory (3) has this given the technical difficulties due to the rapid reaction resistance to CO uptake. Using the O2-dependent term for DLCO/\(V_C\) using single-breath estimates (4, 7), perhaps because of alteration in Dm or Vc.

However, if the reaction resistance is calculated as figure of 0.48, which is close to their estimate (0.4). CO uptake from our data of 1–2/(DLNO/DLCO) gives a resistance and obtained the reaction resistance/total transfer resistance.

All the groups have assumed that \(\theta_{NO}\) is independent of alveolar PO2, although there is no in vitro evidence for this given the technical difficulties due to the rapid reaction of NO and O2. However, our laboratory (3) has previously found some O2 variability of DLNO over the physiological PO2 range and presumed this to be due to alteration in Dm or Vc.

Values for DLNO-to-DLCO ratio and the reaction resistance. The ratio of DLNO to DLCO varies between 3.9 and 4.8, depending on which estimates of DLCO are used. These estimates are rather lower than those made using single-breath estimates (4, 7), perhaps because of the exercise-induced increase in DLCO.

Schuster and Heller (9) extended Guenard’s formula and obtained the reaction resistance/total transfer resistance = DLCO/\(\theta_{CO}V_c\) = 1–2/(DLNO/DLCO). With the use of Heller and Schuster’s formula (9), which assumes that \(\theta_{NO}\) is infinity, the reaction resistance to CO uptake from our data of 1–2/(DLNO/DLCO) gives a figure of 0.48, which is close to our estimate (0.4). However, if the reaction resistance is calculated as DLCO/\(\theta_{CO} \times V_c\) using our value for Vc (Table 3) and using the O2-dependent term for \(\theta_{CO}\) (see calculation of reaction resistance to CO uptake).

\[\text{APPEX}

Subject 1 was 45 yr old and weighed 80 kg. The experiment was performed on a day when the laboratory temperature was 24°C and PaO2 was 752 Torr. Saturated vapor pressure at 37°C was assumed to be 47 Torr.

\[V_T = 2,200 \text{ ml}, \quad RR = 20.3, \quad F_{CO_2} = 4.8\%, \quad F_{CO_2} = 5.7\%, \quad F_{CO} = 0.106\%, \quad F_{CO} = 0.067\%, \quad F_{NO} = 5.260 \text{ ppb}, \quad F_{NO} = 1,467 \text{ ppb}, \quad F_{NO} = 1,200 \text{ ppb}, \quad Hb = 15.3 \text{ g/dl}, \quad COHb = 1\% , \quad metHb = 0.3\%.

Dead space calculations. Anatomical dead space was estimated as 2.2 \times \left[\text{weight (kg) + age (yr)}\right] \times \left[\text{2,200} \div (1,467 + 1,200)\right] = 221 \text{ ml}.

CO2 dead space (6) was calculated from

\[V_D = V_T(F_{CO_2} - F_{CO_2})/F_{CO_2} = 2,200 \times (5.7 - 4.8)/5.7 = 347 \text{ ml}\]

Diffusing capacity calculation for DLNO. Using predicted anatomic dead space (A DCO 1 in Bates et al.)

\[D_{LCO} = V_T \times RR \times (V_T - V_D) \times (F_T - F_d)/(F_e \times V_T - F_t \times V_d) \times (P_b - 47) = 2,200 \times 20.3 \times (2,200 - 221)\]

Using CO2 dead space ([e] in Bates et al.)

\[D_{LCO} = V_T \times RR \times (V_T - V_D) \times (F_T - F_d)/(F_e \times V_T - F_t \times V_d) \times (P_b - 47) = 2,200 \times 20.3 \times (2,200 - 347)\]

\[\times 273/(273 + 24) \times (0.0106 - 0.00067)/(0.00067 \times 2,200 - 0.000106 \times 221) \times (752 - 47) = 36.2 \text{ ml} \times \text{min}^{-1} \times \text{Torr}^{-1}\]
Diffusing capacity calculation for $D_{\text{LNO}}$. Using the predicted anatomic dead space for NO

$$D_{\text{LNO}} = V_t \times RR \times (V_t - V_d) \times ((F_i - F_e)(F_e \times V_t - F_i \times V_d))$$

$$\times (P_b - 47) = 2,200 \times 20.3 \times 273/(273 + 24) \times (2,200 - 221)$$

$$\times (5,260 - 1,467) \times 10^{-9}/(2,200 \times 1,467 - 5,260 \times 221)$$

$$\times (752 - 47) \times 10^{-9} = 211.9 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$$

Using end-tidal $F_{\text{ENO}}$ from the expired NO curve (Fig. 2) as an estimate of $F_{\text{ANO}}$

$$D_{\text{LNO}} = V_t \times RR \times [(F_i - F_e)(F_e \times V_t - F_i \times V_d)] \times (P_b - 47)$$

$$\times 20.3 \times 273/(273 + 24) \times (5,260 - 1,467)$$

$$\times 10^{-9}/1,200 \times (752 - 47) \times 10^{-9} = 184.1 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$$

For dead space and $D_l$ calculations, the respiratory quotient was assumed to be 1 (see RESULTS).

**Calculation of $D_m$ and $V_c$.** Calculations were made as follows

$$D_{\text{mNO}} = (\theta_{\text{NO}} - 2 \cdot 0_{\text{CO}})/(\theta_{\text{NO}}/D_{\text{LNO}} - \theta_{\text{CO}}/D_{\text{LCO}}) \quad (A5)$$

$$D_{\text{mCO}} = D_{\text{mNO}}/2 \quad (A6)$$

$$V_c = 1/(\theta_{\text{CO}}/D_{\text{LCO}} - \theta_{\text{CO}}/D_{\text{mCO}}) \quad (A7)$$

For derivation of these equations, see Ref. 3. The value of $\theta_{\text{NO}}$ (5) was taken as $4.5 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1} \cdot \text{ml}^{-1} (1,500 \text{ mmol} \cdot \text{min}^{-1} \cdot \text{kPa}^{-1} \cdot \text{liter}^{-1})$ and $\theta_{\text{CO}}$ (7) calculated from $1/\theta_{\text{CO}} = 1.3 \times 10^{-9} + 4.1 \times 10^{-8} (P_{\text{CO}}) \text{ min} \cdot \text{Torr}^{-1}$. $P_{\text{CO}}$ was taken as 100 Torr. For both CO and NO, correction for Hb was made by multiplying $\theta$ by Hb $\times (1 - \text{COHb} - \text{metHb})/14.6$.

For subject 1, we used the estimate of $D_{\text{LCO}}$ using the CO2 dead space and $D_{\text{LNO}}$ using end-tidal NO as an estimate of alveolar NO

$$\theta_{\text{CO}} = 1/[1.3 + 4.1 \times 10^{-9}(100)]$$

$$\times (15.3 - 0.3 - 1)/14.6 = 0.6 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1} \cdot \text{ml}^{-1}$$

$$\theta_{\text{NO}} = 4.5 \times (15.3 - 0.3 - 1)/14.6$$

$$= 4.6 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1} \cdot \text{ml}^{-1}$$

$$D_{\text{mNO}} = (4.6 - 2 \times 0.6)/(4.6/184.1 - 0.6/38)$$

$$= 369.7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$$

$$D_{\text{mCO}} = 369.7/2 = 184.9 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$$

$$V_c = 1/[0.6/29.3 - 0.6/(0.5 \times 517)] = 80.0 \text{ ml}$$

For calculation of $D_{\text{mCO}}$, the assumption (8) is made that $\theta_{\text{NO}}$ is infinity

$$D_{\text{mCO}} = D_{\text{LNO}}/2 = 92 \text{ ml} \cdot \text{min}^{-1} \cdot \text{Torr}^{-1}$$

and $V_c$ was obtained by inserting this value for $D_{\text{mCO}}$ into Eq. 7

$$= 1/[0.6/38 - 0.6/(92)]$$

$$= 108 \text{ ml}$$

**REFERENCES**


